These findings make apparent the need for more detailed electrophysiological analysis of Kv1.2 and will certainly prove useful to clarify the structure-function relationship of voltage gated potassium channels.

2709-Pos Board B479

Positioning and Guidance of the Voltage Sensor S4 Within the Omega/Gating-Pore in the Shaker K-Channel

Claudia Lehmann, Hansjakob Heldstab, Nikolaus G. Greeff.
Biophysics Institute Greeff, Uetikon am See, Switzerland.

Inwardly leaking omega-currents in resting Shaker K-channels were first found when the long charged residue R362 on S4 was made short, creating a pore in the voltage-sensing domain (Tombola et al., 2005, 2007). We recently demonstrated that in fact a pair of adjacent charged residues on S4 must be short to build a double-gap (Gamal El-Din et al., 2010). These residues located at every third position A359, R362, R365, R368, R371, K374 can be seen as a rail, shorthand notation ''aRRRRK''. Omega-currents were obtained for the double-gap constructs ''asRRRK'', ''RssRRK'' and ''aRssRK'' (short residues lower case). In a mechanistic view, the long residues slide like bolts in a guidance groove which becomes the leaking omega-pore when two occluding bolts are shortened.

Presently, we study the wall and length of this guidance groove. Especially, we checked in the closed state of S4 whether at the outer end of the groove, E283 on S2 opposes A359 on S4, and whether E293, F290 form the inner end and oppose R362 on S4. Firstly, we kept the inner part open (R362S) and studied different mutant pairs at positions 283(S2) and 359(S4). The size of the resulting omega-current clearly corresponded to the cleft width obtained from molecular modeling. Secondly, leaving the outer part open with A359, we demonstrated that the omega-currents now depended on the cleft width between residues at 362(S4) and 293, 290 on S2.

In conclusion, the omega-pore represents a guidance groove for the gating charges of S4. E283 and E293 located at the outer and inner end of the groove determine the length of the membrane voltage drop. This length also guarantees that during gating always at least one residue senses the field in the pore.

2710-Pos Board B480

The Silent K^+ Channel Subunit, $K_V6.4$. Influences the Gating Charge Movement of $K_V2.1$ in a Heterotetrameric Channel Complex

Elke Bocksteins[1,2], Alain J. Labro[1], Dirk J. Snyders[1], Durga P. Mohapatra[2].
[1]University of Antwerp, Antwerp, Belgium, [2]University of Iowa, Iowa City, IA, USA.

Voltage-gated K^+ (Kv) channels are tetramers of α-subunits that detect changes in membrane potential (V) by a positively charged (Q) voltage-sensing domain (VSD). Molecular movements of VSDs lead to charge displacement that can be recorded as transient gating currents (I_Q), which subsequently results in channel gating. The silent Kv subunit, Kv6.4, does not form functional homotetramers; however, it can tetramerize with Kv2.1 subunits to form functional Kv2.1/Kv6.4 heterotetramers, with a proposed 3:1 stoichiometry. Previously we showed that Kv6.4 subunits exert a significant (~40 mV) hyperpolarizing shift in the voltage-dependent inactivation of heterotetrameric Kv2.1/Kv6.4 channels, as compared to Kv2.1 homotetramers, without significant effects on activation gating. However, the underlying mechanism remains unclear. To address this we analyzed ionic and I_Q recordings from heterotetrameric Kv2.1/Kv6.4 channels transiently expressed in HEK293A cells. Half-maximal displacement of gating charge ($Q_{1/2}$) for Kv2.1 homotetramers was −26 mV, as determined from the charge-voltage (Q-V) curve. Analysis of the decay time constant of $I_{Q\text{-}ON}$ as a function of voltage resulted in a bell shaped curve with a maximal time constant around the midpoint potential of −20 mV. Co-expressing Kv6.4 with Kv2.1 resulted in earlier charge movement as evident from a ~16 mV hyperpolarizing shift in the Q-V curve. Furthermore, we observed a double bell shaped curve for the decay time constant, with maximal time constants around −20 mV and −70 mV; the latter corresponding to the Kv6.4-induced ~40 mV hyperpolarizing shift in the voltage-dependence of channel inactivation. Therefore, we suggest that this more negatively located ON-gating component presumably reflects the voltage-dependence of the Kv6.4 subunit within the Kv2.1/Kv6.4 heterotetramer, and that the VSD movement of only the Kv6.4 subunit is sufficient to induce closed state channel inactivation.

2711-Pos Board B481

Use of Resonance-Wavelength Grating Optical Biosensors to Detect Channel-Protein Interaction in Slack KNa Channels

Matthew R. Fleming, Maile R. Brown, Leonard K. Kaczmarek.
Yale University, New Haven, CT, USA.

Na+-activated potassium (KNa) channels encoded by the Slack and Slick genes contribute to neuronal adaptation during sustained stimulation and regulate the accuracy of timing of action potentials. Activation of protein kinase C (PKC) increases the amplitude of Slack-B currents and slows their rate of activation. Slack protein is known to interact with a variety of cytoplasmic signaling molecules. Using resonance wavelength grating optical biosensors (the SRU Biosciences BIND system), we have determined that direct pharmacological activation of Slack channels by bithionol produces a sustained decrease in mass distribution close to the plasma membrane, and that phosphorylation of Slack channels mimics this decrease in mass. These results were obtained using transfected HEK293 cells, and confirmed in mouse primary cortical neurons. These changes in mass distribution appear to be specific to the Slack channel because pharmacological activation of the very closely related Slick channel with bithionol does not produce a change in mass distribution. Blocking ion flux through the Slack channel during channel activation does not attenuate this response, indicating that ion flux is not necessary for the change in mass. The very C-terminal domain of Slack has been previously shown necessary for channel-protein interactions, and deletion of this region abolished the observed signal. To determine which proteins or signaling molecules are translocating from the plasma membrane upon channel activation, an RNAi screen against probable channel binding partners was performed, and the Protein Phosphatase 1 (PP1) inhibitor Phactr1 was found to be necessary for this decrease in mass. We hypothesize that activation of Slack by either bithionol or phosphorylation leads to the dissociation of Phactr1 with PP1 from the channel complex, allowing the Slack channel to remain in its phosphorylated and active state.

2712-Pos Board B482

The Potassium Delayed Rectifier Conductance in the Sarcolemma and the Transverse Tubular System Membranes of Mammalian Skeletal Muscle Fibers

Julio L. Vergara, Marino DiFranco.
UCLA School of Medicine, Los Angeles, CA, USA.

The transverse tubular system (TTS) plays a key role not only in mediating the mechanism of excitation-contraction coupling, but also in determining the electrical properties of mammalian skeletal muscle fibers. We investigated the properties and distribution (between sarcolemma and TTS membranes) of the K delayed rectifier conductance (g_{KV}) by simultaneously recording fluorescence transients and ionic currents (I_{KV}) from FDB muscle fibers stained with the potentiometric indicator di-8-ANEPPS and voltage-clamped using a two-microelectrode configuration. Enzymatically dissociated fibers were mounted on the stage of an inverted fluorescence microscope equipped with a 460-500//500//513-558nm cube. The external solution had (in mM): 150 LiCl, 4 KCl, 20 MOPS, 2 $CaCl_2$, 1$MgCl_2$ and 10 glucose. The Na, ClC-1, Ca and K_{IR} currents were blocked by external TTX (0.001), 9-ACA (0.4), nifedipine (0.02) and Rb (5), respectively. The membrane capacitance was measured after rendering the fibers electrically passive by replacing external Li and K by TEA. We found that I_{KV} records display a delayed onset and decayed markedly during long depolarizing pulses (400ms) due to inactivation and accumulation mechanisms. Furthermore, while di-8-ANNEPS transients recorded from electrically passive fibers displayed quasi-rectangular kinetic properties, transients recorded from control fibers in the presence of I_{KV} were associated with time-dependent attenuations that matched the kinetics of activation and decay of I_{KV} records. Radial cable model simulations were used to evaluate the voltage-dependent kinetic parameters of g_{KV}, to calculate the rate of accumulation of K^+ ions in the lumen of the TTS, and to determine that the relative distribution of this conductance between the surface and TTS membranes is close to equal. This work was supported by NIH grants AR047664, AR041802, and AR054816.

2713-Pos Board B483

Enhancement of Closed-State Inactivation and ER Retention of Kv4.3 Mediated by N-Terminal KIS Domain of Auxiliary KChIP4A

Yiquan Tang, Ping Liang, KeWei Wang.
Peking University, Beijing, China.

Auxiliary KChIP4a shares high homology of conserved C-terminal core region with other members of Kv channel-interacting proteins (KChIPs), but exhibits a distinct modulation on Kv4 current expression and gating. It has been shown that the unique N-terminus of KChIP4a functions as K^+ channel inactivation suppressor (KIS) that leads to slow inactivation and current inhibition. However, the mechanism by which the KIS domain causes current reduction remains unknown. In this study, we identified a hydrophobic ER-retention motif within the KIS domain of KChIP4a that suppresses Kv4.3 surface expression using confocal imaging and cell surface biotinylation assay. Further dissection of KIS domain revealed several key residues that cause reduction of Kv4.3 peak current, but do not affect surface expression. Examination of gating properties of Kv4.3 co-expressed with either KChIP4a or its core without the